Novel molecular mechanisms of disease susceptibility in plants -- an FTIR study of *Arabidopsis thaliana*

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INTRODUCTION

Understanding the molecular basis of plant resistance to fungal diseases will contribute to reducing world-wide crop losses. One model organism for studying disease resistance is *Arabidopsis thaliana*, a small member of the mustard family for which complete genomic sequence information was publicly released in 2000. *Erysiphe cichoracearum*, the causative agent for powdery mildew disease in a wide range of plants, colonizes and eventually overtakes a host if three events occur. *Erysiphe* spores are carried on the wind, and when they land on the aerial portions of a host plant, they must invade an epidermal (outer) cell, and establish a feeding structure to divert plant nutrients. The fungus must "fly under the radar" of the host's defense responses, which would, if fully activated, quickly kill the invading fungus. Finally, since the fungus is not a saprophytic pathogen (cannot survive on dead tissues), it must keep the host's cells alive until its life cycle is complete. Several genetic loci conferring *p*owdery *m*ildew *r*esistance (*pmr*1-4) have previously been described by Vogel and Somerville [1]. While many disease-resistance pathways involve sensing of salicylic acid and/or jasmonic acid, several genes that operate independently of these hypersensitive responses have been identified; the mutant described below represents a novel form of disease resistance based upon loss of a gene required during a compatible interaction, rather than the action of known host defense pathways.

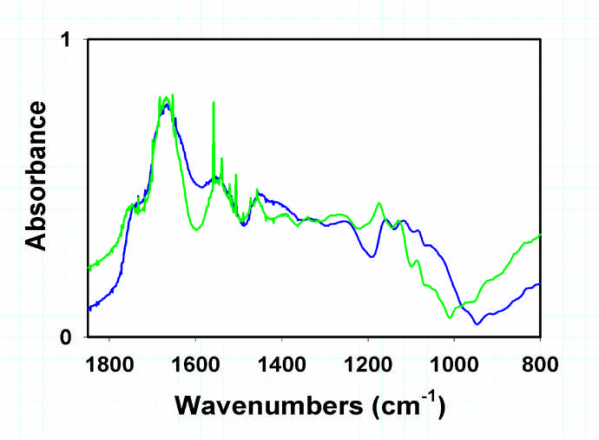
METHODS

An *Arabidopsis* disease-resistance gene named *PMR*6 has recently been cloned and characterized and encodes a pectate lyase-like enzyme with a novel C-terminal domain [2]. Although the protein has been purified, it has resisted all efforts at demonstrating enzymatic activity towards cell wall polysaccharides. Utilizing Beamline 1.4.3, we undertook a comparative FTIR microscopic study of cleared leaves from wild type (WT) Columbia plants of *Arabidopsis*, as well as plants homozygous for a loss-of-function mutation in *pmr*6. We reasoned that large scale modifications in the pectin component of the cell wall in the mutant compared to wild type should be instructive as to the nature of the molecular lesion. IR spectromicrosopy at BL 1.4.3 allows the chemical characterization of distinct cell types in both living and chemically-cleared tissues at a spatial scale of 6 μm x 10 μm, a significant improvement over thermal IR sources. For these experiments, a few dozen matched seedlings of Columbia WT and *pmr*6-1 were germinated on 1.5% agar plates supplemented with 0.5x MS medium, and cultivated in

growth cabinets at 21^{o} C with {16:8} photoperiod. After 2 weeks, the seedlings were transplanted to a peat:perlite mix supplemented with slow-release fertilizer, and grown an additional 10-12 days in a walk-in growth chamber with continuous light. At the early bolting stage, rosette leaves of both treatments were cleared in 1:1 chloroform:methanol and air-dried flat overnight on microscope slides under sterile cover slips. IR spectra were collected (upon removal of the cover slips) in single-beam reflectance mode at 2 cm^{-1} resolution over the range of 4000 - 650 cm^{-1} (2.5 to 16 micron wavelength) with 512 spectra co-added for Fourier transforms. Spectra were then converted to absorbance basis by ratioing to a gold-coated reflection standard, and small amounts of CO_2 and water vapor subtracted from the spectra. At least a dozen (biological replicates) plants from each class were compared.

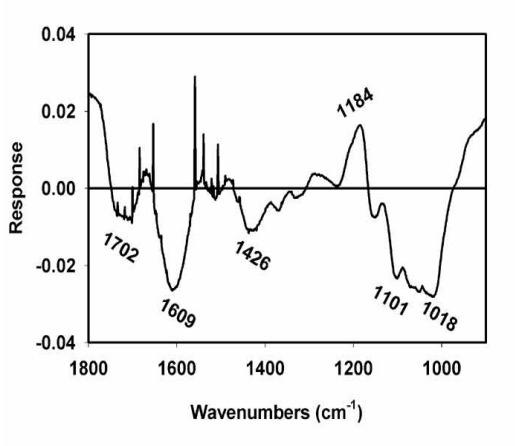
For initial data exploration, spectra were converted from Nicolet's OMNIC software controlling the microscopes at BL 1.4.3, to the JCAMP.DX format, and analyzed after area-normalization using **Win DAS** [3], a widely used multivariate statistical package in biotechnology and biospectroscopy. For extremely multivariate data sets such as those produced in FTIR or NMR spectroscopy, the very first step in rational data exploration occurs at the level of dimensional reduction. One such method, principal components analysis (PCA, also known as singular-value decomposition) seeks a new set of m axes (<< r, the number of variates in each spectrum) that projects as much of the variance in the original data as possible. Using a covariance matrix, the software initially calculates a ranking (from increasing to decreasing variance) of the top ten vectors that "span" the original data. In most spectral applications, the first two or three PCs explain much of the difference between the 'treatment' and 'control', often highlighting spectral contributions (known as PC 'loadings' or 'scores') hard to see in the original spectra. Below we discuss two of the products of such an analysis to interpret the molecular distinction between wild type *Arabidopsis* plants and those carrying the disease resistance lesion. It should be kept in mind however, that chemometrics methods are quite general, and can be used, e.g. for interpretation of remote sensing data, as well as DNA microarray experiments in clinical medical settings.

RESULTS

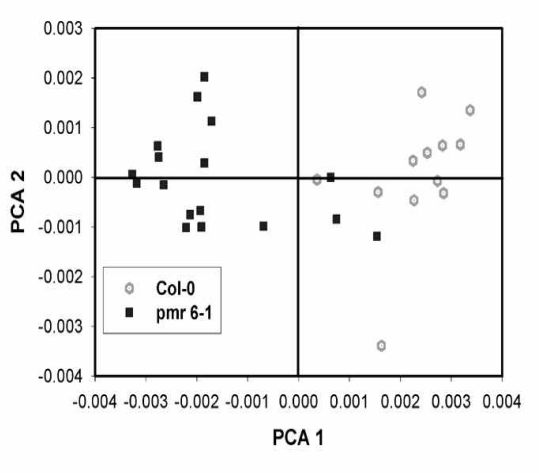


To determine if mutations in PMR6 altered cell wall composition, FTIR spectra were acquired at BL 1.4.3. **Figure 1** compares the mean absorbance spectra of a dozen WT leaves (green) and eighteen *pmr6* leaves (blue) in the carbohydrate 'fingerprint' region from 1800-800 cm^{-1}. Visual inspection of the absorbance spectra from *pmr*6 leaves reveals somewhat greater absorbance in the region attributed to pectin, a major class of cell wall polysaccharides in plants. This is not surprising given that *pmr*6 may be a pectin-degrading enzyme. The absorbance peaks

attributed to cellulose in *pmr*6 cell walls shift down in energy, indicating a greater degree of hydrogen-bonding than WT.



Principal components analysis was employed to identify features that differ between *pmr*6 and wild type, but that are not obvious in the raw spectra. The first three PCs explained 64%, 19% and 9%, respectively of the variation in the full data set. The signature peaks (1609, 1101, 1018 cm^{-1}) of the of the first principal component (**Figure 2A**) points to the enrichment of the of the *pmr*6 cell wall in pectins with a lower degree of esterification and that *pmr*6 cellulose has more intermolecular hydrogen bonds (1426 cm^{-1}) than WT. The 2nd PC (not shown) corresponded to higher levels of protein in the *pmr*6 leaves relative to WT. The statistical separation of the two types of *Arabidopsis* plants can be seen in the biplot of **Figure 2B**.



Spectroscopic observation of the altered pectin composition of the cell wall fits well with the possible pectin-degrading/binding activity for the *PMR6* gene. The alterations of seen by FTIR in *pmr*6 cell wall composition may have made the plants less palatable to the pathogen *Erysiphe* spp. We are at work to identify other actors in this pathway and to interpret changes in cell wall composition by various IR techniques at the ALS.

REFERENCES

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